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Effect of Alzheimer Caregiving Stress and Age on Frailty Markers Interleukin-6, C-Reactive Protein, and D-Dimer

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Background. Elevated plasma levels of interleukin (IL)-6, C-reactive protein (CRP), and D-dimer belong to the biological alterations of the ''frailty syndrome,'' defining increased vulnerability for diseases and mortality with aging. We hypothesized that, compatible with premature frailty, chronic stress and age are related in predicting inflammation and coagulation activity in Alzheimer caregivers.

Methods. Plasma IL-6, CRP, and D-dimer levels were measured in 170 individuals (mean age 73 ± 9 years; 116 caregivers, 54 noncaregiving controls). Demographic factors, diseases, drugs, and lifestyle variables potentially affecting inflammation and coagulation were obtained by history and adjusted for as covariates in statistical analyses.

Results. Caregivers had higher mean levels of IL-6 (1.38 ± 1.42 vs 1.00 ± 0.92 pg/mL, $p = .032$) and of D-dimer (723 ± 530 vs 471 ± 211 ng/mL, $p < .001$) than controls had. CRP levels were similar between groups ($p = .44$). The relationship between caregiver status and D-dimer was independent of covariates ($p = .037$) but affected by role overload. Age accounted for much of the relationship with IL-6. After controlling for covariates, the interaction between caregiver status and age was significant for D-dimer ($\beta = .20$, $p = .029$) and of borderline significance for IL-6 ($\beta = .17$, $p = .090$). Post hoc regression analyses indicated that, among caregivers, age was significantly correlated with both D-dimer ($\beta = .50$, $p < .001$) and IL-6 ($\beta = .38$, $p = .001$). Among controls, however, no significant relationship was observed between age and either D-dimer or IL-6.

Conclusions. The interaction between caregiving status and age for D-dimer and IL-6 suggests the possibility that older caregivers could be at risk of a more rapid transition to the frailty syndrome and clinical manifestations of cardiovascular diseases.

PROVIDING care to a spouse who is suffering from Alzheimer's disease confers a significant health risk (1). Dementia caregivers have higher rates of hypertension (2) and coronary artery disease (CAD) (3), and they also show slower wound healing (4) than do people in the community who are not caregivers. Moreover, all-cause mortality is higher in caregivers than in controls (5). Increased morbidity and premature death in caregivers suggest that caregiving strain could hasten the transition to the ''frailty syndrome'' that is linked to a range of physiological alterations which arise during the process of aging (6).

Frailty designates a decline in health function that is accompanied by an increase in disease vulnerability and mortality (7). The proinflammatory cytokine interleukin (IL)-6, the acute phase reactant C-reactive protein (CRP), and the procoagulant molecule D-dimer all increase with age, and elevated plasma levels of these three measures have also been identified as biological accompaniments of frailty (8,9). Whereas the pleiotropic biological activity of IL-6 highlights its role in many different diseases of older age (10), activities of CRP and D-dimer are more intimately related to CAD (11,12).

There is a strong interplay between inflammation and coagulation in arterial vascular pathology (13). Population studies found associations between IL-6 and D-dimer (14) and between CRP and D-dimer (14,15). On a mechanistic level, IL-6 stimulates hepatic synthesis of CRP (16) and D-dimer stimulates monocytes to produce IL-6 in vitro (17). A recent review of data from the Cardiovascular Health Study (18) concluded that IL-6, CRP, and D-dimer might play a particularly important role in atherosclerosis development in elderly persons (19–21).

Women who are Alzheimer caregivers have higher levels of IL-6 than do noncaregiving control women (22), and caregiving strain has been prospectively associated with an increase in plasma IL-6 levels in Alzheimer caregivers of both sexes (23). In a preliminary study on a subset of individuals investigated in the present study, we reported higher D-dimer in caregivers than in gender-matched controls (24). Therefore, low-grade systemic inflammation and coagulation activation in response to caregiving strain provides one biological pathway through which caregivers might age more rapidly and as a consequence manifest, for example, cardiovascular disease (2,3) at a younger age than noncaregivers might. It is unknown, however, whether the burden of caregiving and increased age are related in predicting the level of systemic inflammatory and coagulant processes. Their being related would imply that caregiver stress and age interact in accelerating the transition to the frailty syndrome. Although cross-sectional, such an obser-

vation might inspire prospective research to possibly confirm the hypothesis that markers of frailty predict cardiovascular morbidity and mortality in dementia caregivers several years down the line.

We compared IL-6, CRP, and D-dimer in 116 Alzheimer caregivers and 54 noncaregiving controls and controlled for demographic factors, diseases, drugs, and lifestyle variables known to affect inflammation and coagulation. We also assessed levels of general psychological distress, role overload, and social support to confirm our a priori assumption that caregivers are more stressed than are controls. Our first aim was to confirm previous findings of increased IL-6 (22) and D-dimer (24) in caregivers. We further hypothesized that CRP would be higher in caregivers than in controls. Our second aim was to investigate the role of age in the relationship between caregiving strain and frailty indices. We hypothesized that age would mediate the relationships between caregiving strain and frailty markers or that age would interact with caregiver status in predicting frailty markers.

Methods

Participants

Venous blood to measure D-dimer, IL-6, and CRP was obtained from 170 participants (116 caregivers, 54 controls; 51 men, 119 women; 93% Caucasian) who participated in a longitudinal study on effects of dementia caregiving stress on health. We report cross-sectional data obtained after completion of the study's enrollment period. All participants were recruited from the University of California at San Diego (UCSD) Research Centers, through community support groups or through physician referrals. They gave written informed consent to the study protocol, which was approved by the UCSD Institutional Review Board. All caregivers were spouses of patients with Alzheimer's disease who lived with and provided informal in-home care for the patient. The control group consisted of community-dwelling elderly men and women not providing care to a household member but also living with their spouse. Controls were recruited in the same proportion of gender as caregivers through a variety of means including community outreach, advertising, referrals from study participants and the community, and collaborations with the UCSD Alzheimer Disease Research Center. Controls were not excluded for stressful life events or any other reasons in order that a representative sample of noncaregivers could be studied. All participants had to be $\geq$ 55 years old and free of anticoagulant medication.

Medical Data

At participants' homes, a research nurse conducted a structured medical history that asked about past and present diagnoses of diseases and treatment including any medications as well as health habits. Here, we specifically report on health characteristics, which may affect inflammation and coagulation. Smoking status was specified in terms of never smokers, former smokers, and current smokers with the latter group also indicating the average number of cigarettes currently smoked per day. However, only 6% of participants (7 caregivers and 3 controls) were current smokers. The proportion of current smokers ($p = .92$) and the number of cigarettes currently smoked daily (caregivers 1.0 $\pm$ 4.8, controls 0.1 $\pm$ 0.6; $p = .81$) were not different between groups. Therefore, the number of cigarettes smoked per day was not used for further analyses, and smokers were categorized into current or former smokers ($n = 78$) and never smokers ($n = 86$). Alcohol consumption was quantified in terms of the number of drinks of alcohol that participants consumed in an average week during the last 6 months. Physical exercise was quantified in terms of the number of days participants performed physical exercise in an average week during the last 6 months. Medical data were complete for all 170 caregivers and controls except for reports on smoking status ($n = 164$), alcohol consumption ($n = 157$), and physical exercise ($n = 158$).

Body mass index (BMI) was computed as the ratio between weight in kilograms and height in square meters. For statistical analyses, cancer was defined as a positive history of any current or lifetime cancer diagnosis. Cerebrovascular disease was defined as a positive history for stroke or transient ischemic attack. CAD was considered positive if there was a history of myocardial infarction, angina, heart attack, or coronary artery bypass surgery. Platelet aggregation–inhibiting medication consisted of aspirin, various types of nonsteroidal anti-inflammatory drugs, ticlopidine, or clopidrogel. The different sorts of antihypertensives (e.g., diuretics, calcium channel blockers), statins, hormone replacements (estrogen replacement or combined estrogen and progesterone replacement), and antidepressants (e.g., selective serotonin reuptake inhibitors, tricyclics) were grouped in one category each.

Psychosocial Measures

Caregivers and controls were asked to complete a set of self-rated questionnaires when the nurse visited them at their homes. We used the Brief Symptom Inventory (BSI) global severity index to asses the extent to which participants felt global psychological distress (25). For the BSI, participants are asked to rate how much each of 53 items ''had caused distress during the past six months, including today.'' Response choices range from 0 (not at all) to 4 (extremely). Average responses to different subscales are used to calculate an overall score ranging between 0 and 4. Pearlin's Role Overload scale (26) was used to assess the extent to which caregivers and controls felt overwhelmed or overloaded by life responsibilities. The scale consists of 4 items rated from 1 (not at all) to 4 (completely); for example, ''you work hard (as a caregiver) but never seem to make any progress.'' The sections in parentheses specific to caregivers were excluded in the questionnaires given to noncaregiving controls. The scale has demonstrated good internal reliability (Cronbach's alpha = .80). For the present study, average responses to each item were used to create an overall overload score (range, 1–4). Perceived social support was measured by Pearlin's Expressive Support Scale (26) asking eight questions (Cronbach's alpha = .87) with responses ranging from 1 (strongly disagree) to 4 (strongly agree) providing an average support score (range, 1–4). Twelve participants had missing data for BSI global sever-

ity index, whereas data on role overload and on social support were complete in all participants.

Biological Assays

At each participant's home, venous blood was drawn through a 22-gauge venous forearm catheter. Blood for the D-dimer assay was dispensed into polypropylene tubes containing 3.8% sodium citrate (9:1, vol/vol) and spun at 2000 g for 10 minutes at room temperature. Blood for the IL-6 and CRP assays was dispensed in EDTA tubes and spun at 3000 g for 10 minutes at 4–8°C. Obtained plasma was immediately stored in plastic tubes at –80°C until analyzed.

Plasma D-dimer levels were measured by an enzyme-linked immunosorbent assay (Asserachrom Stago, Asnières, France). Plasma levels of IL-6 were measured by a high-sensitivity immunoassay kit (Quantikine, R&D Systems, Minneapolis, MN). Measurement of plasma CRP levels was taken by using the High Sensitive CRP Reagent Set (DiaSorin, Stillwater, MN) using the Roche Cobas Mira Plus analyzer (Roche, Palo Alto, CA). Intra- and inter-assay coefficients of variation were <10% for all analyses. Because of occasional assay problems, data were missing for IL-6 in 6 participants (5 caregivers, 1 control), for CRP in 11 participants (9 caregivers, 2 controls), and for D-dimer in 8 participants (6 caregivers, 2 controls).

Statistical Analyses

Data were analyzed using the SPSS statistical software package (version 13.0; Chicago, IL). All testing was two-tailed with the significance level set at $p < .05$. Because of a skewed distribution, values for BMI, alcohol consumption, physical exercise, IL-6, CRP, D-dimer, BSI global severity index, and role overload were all logarithmically transformed before statistical analyses. After logarithmic transformation, IL-6, CRP, and D-dimer data showed a normal distribution (Kolmogorov–Smirnov test) with all values for CRP and IL-6 being within 3 standard deviations (*SD*) from the mean and with the highest D-dimer value being <3.2 *SD* from the mean. Data are presented as mean ± *SD* or geometric mean with 95% confidence interval (CI) or both in the text and tables, and as log-transformed value in the figure.

Pearson and Spearman correlation coefficients were estimated for continuous and categorical variables, respectively. To calculate differences in the frequency of categorical variables between caregivers and controls, we used chi-square testing applying Pearson's Test or Fisher's Exact Test whenever appropriate. We used analyses of variance (ANOVA) to test for a difference in continuous measures (e.g., IL-6, CRP, and D-dimer) between caregivers and controls and analyses of covariance (ANCOVA) to adjust these differences for the various health characteristics. Categorical variables (e.g., use of antihypertensive drugs) were treated and entered as dummy variables (0=no, 1=yes). In a first step, we adjusted for demographic data, health habits, cardiovascular risk factors and diseases, and the various medication types to test whether the relationship between caregiver status and frailty markers would be independent. In a second step, we further considered emotional support, general psychological distress, and levels of role overload as covariates to investigate whether psychosocial measures would additionally affect this relationship. Post hoc analyses were by Fisher's Least Significant Difference.

Table 1. Characteristics of 170 Study Participants

Health Factor	Caregivers ($N = 116$)	Controls ($N = 54$)	p Value
Age, y	72.9 ± 8.7	67.6 ± 6.8	<.001
Female	68%	74%	.429
Body mass index, kg/m^2	25.4 ± 4.3	25.8 ± 4.7	.502
Never/current or former smoker	51%/49%	55%/45%	.686
Alcohol consumption, drinks/wk	4.25 ± 5.77	4.35 ± 5.31	.339
Physical exercise, d/wk	2.19 ± 2.36	2.98 ± 2.46	.033
Hypercholesterolemia	35%	37%	.830
Arterial hypertension	35%	21%	.142
Type II diabetes	3%	6%	.681
Cancer	30%	22%	.281
Cerebrovascular disease	3%	0%	.308
Coronary artery disease	6%	4%	.721
Platelet antiaggregant drug	38%	30%	.292
Antihypertensive drug	47%	30%	.037
Statin medication	17%	28%	.114
Hormone replacement therapy	36%	48%	.139
Antidepressant drug	25%	17%	.225
General distress	0.46 ± 0.42	0.30 ± 0.25	<.001
Role overload	2.30 ± 0.81	1.54 ± 0.51	<.001
Social support	1.78 ± 0.50	1.67 ± 0.43	.172

Note: Data are given as means ± standard deviation or percentage values.

We also tested whether caregiver status would interact with age in predicting frailty markers. For this purpose, we performed linear regression analyses with frailty markers as dependent variables and caregiver status, age, the interaction between caregiver status and age, and covariates as independent variables (27). From these analyses, we only report on interaction terms to prevent redundancy with findings from ANCOVA. A significant interaction between age and caregiver status suggests a differential relationship between age and frailty markers for caregivers and controls. To reduce problems resulting from multicollinearity, we centered age values to the mean and defined caregiver status as −1/2 (controls) and +1/2 (caregivers) (28). Collinearity between predictors was tested and found to be tolerable in all analyses.

In ANCOVA and regression analyses, we omitted participants who did not have complete data for all covariates case-wise (i.e., ''listwise'' deletion in SPSS). The 25 participants who did not have complete data in terms of covariates and both D-dimer and IL-6 were not significantly different in any frailty marker and participant characteristics listed in Table 1 from the 145 participants having a complete data set (statistics not shown).

Results

Participants' Characteristics

Table 1 presents participants' characteristics showing that, on average, caregivers were about 5 years older than controls. Also, caregivers reported a higher rate of antihypertensive drug intake and engaged in less physical exercise than controls. The proportions in other variables of health habits, diseases, and drug categories were equally distributed between groups. In terms of psychosocial data, caregivers showed the expected higher levels in general

Table 2. Correlations Between Health Characteristics and Coagulation and Inflammation

Health Factor	IL-6	CRP	D-dimer
Age, y	.30*	.03	.47*
Gender (male = 0, female = 1)	−.03	.17†	−.13
Body mass index, kg/m^2	.24‡	.32*	.06
Former or current smoker (no = 0, yes = 1)	.06	.09	−.09
Alcohol consumption, drinks/wk	−.08	−.03	−.10
Physical exercise, d/wk	−.04	−.11	.12
Hypercholesterolemia (no = 0, yes = 1)	.04	−.15	.05
Arterial hypertension (no = 0, yes = 1)	.14	−.04	.15
Type II diabetes (no = 0, yes = 1)	−.01	.03	.01
Cancer (no = 0, yes = 1)	.05	.03	.12
Cerebrovascular disease (no = 0, yes = 1)	−.08	.08	−.02
Coronary artery disease (no = 0, yes = 1)	.08	.07	.15
Platelet antiaggregant drug (no = 0, yes = 1)	.10	.01	.08
Antihypertensive drug (no = 0, yes = 1)	.18†	.05	.23‡
Statin medication (no = 0, yes = 1)	−.01	−.16†	.04
Hormone replacement therapy (no = 0, yes = 1)	−.12	−.20†	−.21‡
Antidepressant drug (no = 0, yes = 1)	.01	.20†	.06
General distress	.01	.04	.03
Role overload	−.03	.05	.07
Social support	.09	−.01	.16†

Notes: Significance level of correlation coefficients: *$p < .001$; †$p < .05$; ‡$p < .01$.

IL-6 = interleukin-6; CRP = C-reactive protein.

psychological distress and role overload compared with controls, whereas the amount of perceived social support did not differ between groups.

Association of Coagulation and Inflammation With Participants' Characteristics

Table 2 shows the univariate correlation coefficients for the relationships between IL-6, CRP, and D-dimer and the various demographic factors and health characteristics in the entire study population. There were significant bivariate associations between inflammation and coagulation measures with age, gender, BMI, several drug categories, and social support. In contrast, IL-6, CRP, and D-dimer did not significantly correlate with smoking status, disease history, general psychological distress, and role overload.

Associations Between Coagulation and Inflammation Markers

In bivariate correlation analyses, IL-6 correlated with D-dimer ($r = .33$, $p < .001$) and with CRP ($r = .37$, $p < .001$). In addition, CRP showed a trend towards a statistically significant correlation with D-dimer ($r = .15$, $p < .07$).

Differences in Coagulation and Inflammation Markers Between Groups

Multivariate ANOVA showed a significant group (caregiver vs controls) effect ($F_{3,145} = 5.1$, $p = .002$). Table 3 shows that caregivers had higher mean D-dimer levels ($F_{1,160} = 13.7$, $p < .001$) and higher mean IL-6 levels ($F_{1,162} = 4.7$, $p = .032$) than controls, whereas mean CRP levels did not differ between groups ($F_{1.157} = 0.6$, $p = .436$).

We then computed ANCOVA with adjustment for all demographic and medical health characteristics as covariates (cf. Table 3 legend for detailed list of covariates). Caregivers continued to have higher D-dimer than controls

Table 3. Frailty Markers in Caregivers and Controls

Frailty Marker	Analyses of Variance		Analyses of Covariance*	
	Caregivers	Controls	Caregivers	Controls
D-dimer, ng/mL	723 ± 530†	471 ± 211	696 ± 448‡	524 ± 464
	600 (542–665)	429 (370–497)	571 (515–632)	466 (401–542)
	$n = 110$	$n = 52$	$n = 101$	$n = 50$
IL-6, pg/mL	1.38 ± 1.42‡	1.00 ± 0.92	1.26 ± 1.35	1.23 ± 1.39
	1.00 (0.86–1.15)	0.76 (0.62–0.93)	0.92 (0.80–1.08)	0.85 (0.68–1.06)
	$n = 111$	$n = 53$	$n = 101$	$n = 50$
CRP, mg/L	3.20 ± 6.00	2.28 ± 2.88	3.73 ± 5.53	1.85 ± 5.73
	1.57 (1.25–1.96)	1.34 (0.97–1.85)	1.52 (1.21–1.93)	1.36 (0.97–1.92)
	$n = 107$	$n = 52$	$n = 97$	$n = 50$

Notes: Data are given as means ± standard deviation and as geometric means (95% confidence interval).

*Adjusted for age, gender, body mass index, smoking status, alcohol consumption, exercise, hypercholesterolemia, hypertension, diabetes, cancer, cerebrovascular and coronary artery disease, and drugs (platelet inhibiting medication, antihypertensives, statins, hormone replacement therapy, antidepressants).

†$p < .001$; ‡$p < .05$.

IL-6 = interleukin-6; CRP = C-reactive protein.

($F_{18,132} = 4.4$, $p = .037$) with age emerging as the only significant covariate of D-dimer ($F_{1,132} = 21.0$, $p < .001$).

Further adjustment for emotional support ($F_{19,131} = 3.6$, $p = .059$) or general psychological distress ($F_{19,129} = 3.5$, $p = .063$) essentially maintained the difference in D-dimer between groups. However, when entering the level of role overload as a covariate, the difference in D-dimer between caregivers and controls became insignificant ($F_{19,131} = 1.8$, $p = .18$). These analyses suggest that caregivers had higher D-dimer levels than controls had because they had greater role overload, not because they were older than controls.

In contrast, IL-6 levels were no longer different between groups when controlling for demographic and medical health characteristics ($F_{18,132} = 0.4$, $p = .54$). In that model, age ($F_{1,132} = 9.5$, $p = .003$) and BMI ($F_{1,132} = 7.6$, $p = .007$) both emerged as significant covariates of IL-6, suggesting that age accounted for a considerable proportion of the relationship between caregiver status and level of IL-6. Further adjustment for social support, psychological distress, and role overload did not yield a significant association between caregiver status and IL-6 levels. CRP remained also unchanged between groups after adjustment for demographic and health characteristics ($F_{18,128} = 0.3$, $p = .62$); BMI emerged as the only significant covariate of CRP ($F_{1,128} = 20.1$, $p < .001$). Additional adjustment for psychosocial variables did not change these findings.

Role of Age

The above analyses showed that caregivers had higher D-dimer than did controls independent of age; however, the relationship between caregiver status and IL-6 was eliminated when age was included in the model, suggesting that age better accounted for elevated IL-6 than caregiver status. We elected to conduct additional analyses to determine whether age was differentially associated with D-dimer and IL-6 for caregivers and controls. For this purpose, we conducted multiple regression analyses to test for a significant interaction between caregiver status and age in

predicting levels of D-dimer and IL-6, both with and without adjustment for medical and psychosocial covariates.

Crude interaction effect.—Results of our first regression analysis, predicting D-dimer levels, revealed a significant interaction between age and caregiver status ($t = 2.02$, $p = .045$). This interaction indicated that age was differentially associated with level of D-dimer among caregivers and controls. Post hoc analyses using the method described by Holmbeck (27) revealed that age was significantly correlated with D-dimer in caregivers ($\beta = .50$, $p < .001$; $n = 110$; Figure 1A), but not in controls ($\beta = .16$, $p = .31$; $n = 52$; Figure 1B). Results of our regression analysis predicting IL-6 levels revealed a nonsignificant interaction between age and caregiver status ($p = .18$), suggesting that age was similarly related with IL-6 in both groups.

Adjustment for covariates.—Gender, BMI, smoking status, alcohol consumption, exercise, hypercholesterolemia, hypertension, diabetes, cancer, cerebrovascular disease and CAD, platelet antiaggregants, antihypertensives, statins, hormone replacements, and antidepressants were then also entered into regression equations. For D-dimer, the Age × Caregiver status interaction continued to be significant ($t = 2.21$, $p = .029$). Similarly, the interaction remained significant after additional controlling for emotional support, general psychological distress, and role overload ($t = 2.58$, $p = .011$). Post hoc analyses controlling for all demographic, medical, and psychosocial measures showed that age was significantly associated with D-dimer in caregivers ($\beta = .50$, $p < .001$; $n = 99$), but not in controls ($\beta = .03$, $p = .84$; $n = 50$).

The interaction between caregiver status and age was not a significant predictor of IL-6 level after controlling for the same set of demographic and medical covariates ($p = .14$). When emotional support, psychological distress, and role overload were also controlled for, however, the Caregiver status × Age interaction reached borderline significance ($t = 1.71$, $p = .090$). To explore this observation further, post hoc analyses were conducted while controlling for all demographic, medical, and psychosocial variables. Results of these analyses indicated that age was positively associated with IL-6 level among caregivers ($\beta = .38$, $p = .001$; $B = .016$; $n = 99$), but not among controls ($\beta = .04$, $p = .81$; $B = .002$; $n = 50$).

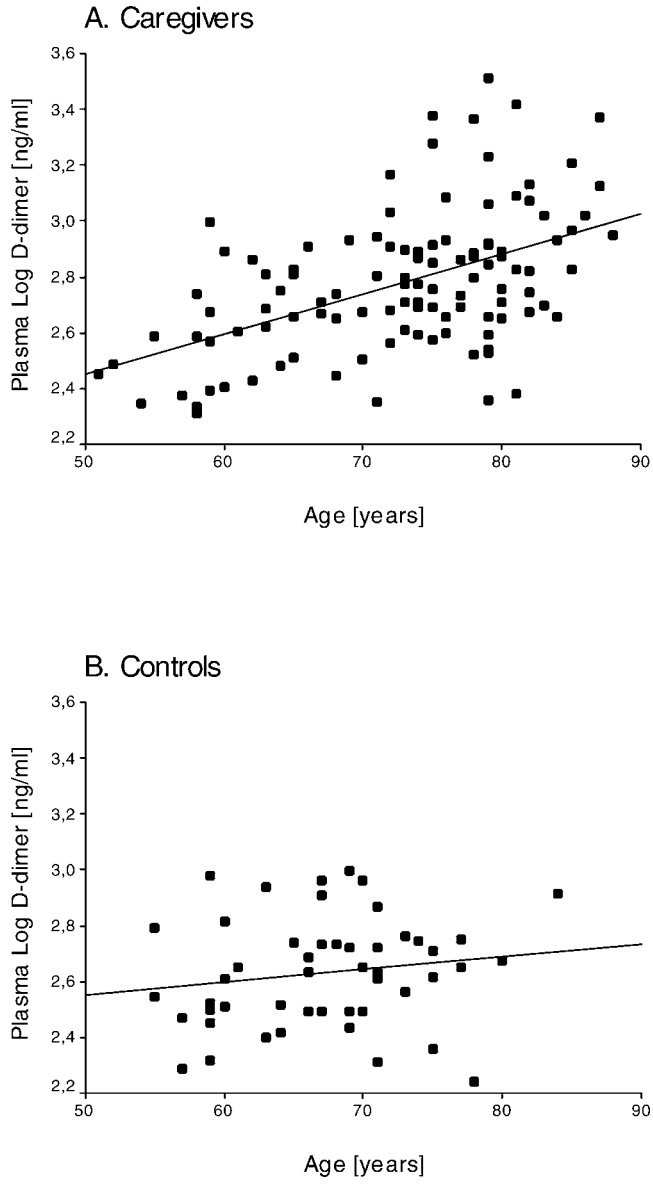

Figure 1. Interaction between age- and log-transformed D-dimer levels in caregivers (**A**) and controls (**B**). The slope of the fit line was steeper in caregivers (unstandardized coefficient B = .014) than in controls (B = .004). Note the identical scaling of the *x* and *y* axes.

Discussion

Confirming previous studies (22,24), crude analyses showed higher plasma levels of IL-6 and D-dimer in Alzheimer caregivers compared to noncaregiving controls. Controlling for various demographic variables (including age) and health characteristics known to affect IL-6 and D-dimer (8,9,29–34), levels of D-dimer but not levels of IL-6 continued to be higher in caregivers than in controls suggesting that caregiving stress uniquely relates to elevated D-dimer. This notion gains support from the observation that caregivers had significantly higher levels of role overload, and controlling for overload rather specifically rendered the relationship between caregiver status and elevated D-dimer nonsignificant. In contrast, general psychological distress did not meaningfully affect the relationship between caregiving status and D-dimer level. In other words, a general state of exhaustion due to the stresses of caregiving (rather than the experience of general psychological distress) may result in coagulation activity in dementia caregivers. Furthermore, although social support appeared to be related to D-dimer level, controlling for social support did not affect the relationship between caregiving status and level of D-dimer, likely due to there being no relationship between caregiving status and social support.

In our previous study that included a subset of 68 individuals also included in the present study, D-dimer was higher in caregivers than in controls (24). Our former analysis was preliminary, however, because of the comparatively small sample size, which did not allow us to reliably control for covariates including age. D-dimer indicates both fibrin formation following activation of the coagulation

cascade and subsequent fibrin degradation by the fibrinolytic system (35). The present findings strongly support our previous notion that fibrin turnover is exaggerated in caregivers. The observation that chronic stress of providing care to a demented spouse confers an increased hypercoagulability risk adds to previous research showing that different types of chronic stressors elicit D-dimer elevation (36).

As was not the case for D-dimer, a significant amount of the relationship between caregiver status and IL-6 was due to age differences between these groups. In addition, the interaction between age and caregiver status significantly predicted D-dimer level and was of borderline significance in predicting IL-6 level. Age was more strongly associated with these two frailty markers in caregivers than in controls independent of all medical and psychosocial covariates. We conclude that psychosocial correlates of caregiving stress other than general psychological distress and role overload might act in the context of age to increase D-dimer level in caregivers. For example, with increasing age, caregivers might become particularly vulnerable to the stresses associated with caregiving, particularly outcomes such as cardiovascular diseases. This assumption is partially supported by a previous study showing a positive association between age and covariate-adjusted 4-year mortality rates (5).

Although chronic stress has been associated with elevated CRP level (37,38), we found no significant difference in plasma levels of CRP between caregivers and controls. Given that the absolute mean CRP value was relatively higher in caregivers, a power issue could be involved. Also, our study was not designed to assess and control for the many immunological and molecular steps modulating the inflammatory cascade leading from stress-related increase in IL-6 to increased production and release of CRP by the liver (16).

IL-6 and D-dimer predict disease states which become more prevalent in aging individuals. For instance, elevated levels of IL-6 play an important pathogenetic role in cardiovascular diseases, osteoporosis, Alzheimer's disease, and hematological neoplasia (10). Elevated D-dimer predicts coronary events (12), recurrence of venous thromboembolic events (39), and poor outcome in patients with cancer (40). Therefore, our observation of increased levels of IL-6 and D-dimer provides one biological pathway by which chronic stress could hasten the development from clinically silent to manifest organic diseases (and ultimately death) in aging Alzheimer caregivers. Our observations further suggest that older caregivers might be particularly vulnerable to experience accelerated health detoriation and transition to frailty. If confirmed in a longitudinal study, our data may suggest that there should be explorations of ways to attenuate caregiving stress in elderly caregivers, particularly, for example, with approaches such as enhancing respite availability, providing practical assistance with caregiving, or modifying cognitive and coping strategies aimed at lessening stress impact.

Our results need to be viewed within the context of several possible limitations. First, there may be unreliability in the health categorization (which were based on self report) of our participants. Although previous studies showed good agreement between medical charts and self-report of diseases and drug intake (41,42), one might argue that health characterization of individuals would be more accurate if obtained from medical records. To the extent that the self-report data created error, there would be no reason to expect it to be unevenly distributed between caregivers and controls; such error variance would tend to lessen the likelihood of finding meaningful relationships among the variables of interest, thus introducing a conservative bias. Second, in terms of generalizing from our results, it is important to note that our participants were primarily Caucasians of middle class economic status. Whether there are effects of race, ethnicity, or more pervasive economic stress on the relationships of caregiving to markers of frailty will need to be determined through studies of larger and more diverse populations. Third, although our sample size allowed controlling for a reasonable set of modifiers of IL-6 and D-dimer levels, a very large sample size would be required to control for all sorts of disease categories and medication common in an elderly study population. There were a number of insignificant differences in medical variables (e.g., frequency in use of statins or hormone replacement therapy) which may not exert substantial effects individually but, when aggregated and applied to a larger sample, might account for some of the differences observed between groups. Also, a more in-depth and costly medical work-up would be required to track diseases such as occult atherosclerosis (43), which may affect frailty markers. We also found in a subset of the present sample that increased apnea hypopnea index was related to greater IL-6 levels and that more Stage 2 sleep was related to greater D-dimer levels, specifically in the caregivers (44). Because we did not investigate sleep in the present study, poor sleep could also contribute to some of the relationship between caregiver stress and increased IL-6 and D-dimer levels, respectively.

Conclusion

Alzheimer caregiver strain is related to markers of low-grade systemic inflammation and coagulation activation, which are also part of the frailty syndrome. Whereas caregiving stress independently predicted D-dimer level, age was a stronger predictor of D-dimer and IL-6 levels in caregivers than in controls. Biological changes observed may help explain accelerated health decline with, for example, premature manifestation of atherosclerotic diseases and mortality in Alzheimer caregivers, particularly in those of older age.

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References

1. Vitaliano PP, Zhang J, Scanlan JM. Is caregiving hazardous to one's physical health? A meta-analysis. *Psychol Bull*. 2003;129:946–972.
2. Shaw WS, Patterson TL, Ziegler MG, Dimsdale JE, Semple SJ, Grant I. Accelerated risk of hypertensive blood pressure recordings among Alzheimer caregivers. *J Psychosom Res*. 1999;46:215–227.

3. Vitaliano PP, Scanlan JM, Zhang J, Savage MV, Hirsch IB, Siegler IC. A path model of chronic stress, the metabolic syndrome, and coronary heart disease. *Psychosom Med.* 2002;64:418–435.
4. Kiecolt-Glaser JK, Marucha PT, Malarkey WB, Mercado AM, Glaser R. Slowing of wound healing by psychological stress. *Lancet.* 1995;346:1194–1196.
5. Schulz R, Beach SR. Caregiving as a risk factor for mortality: the Caregiver Health Effects Study. *JAMA.* 1999;282:2215–2219.
6. Ferrucci L, Cavazzini C, Corsi A, et al. Biomarkers of frailty in older persons. *J Endocrinol Invest.* 2002;25(10 suppl):10–15.
7. Walston J, Fried LP. Frailty and the older man. *Med Clin North Am.* 1999;83:1173–1194.
8. Walston J, McBurnie MA, Newman A, et al. Frailty and activation of the inflammation and coagulation systems with and without clinical comorbidities: results from the Cardiovascular Health Study. *Arch Intern Med.* 2002;162:2333–2341.
9. Cohen HJ, Harris T, Pieper CF. Coagulation and activation of inflammatory pathways in the development of functional decline and mortality in the elderly. *Am J Med.* 2003;114:180–187.
10. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. *Annu Rev Med.* 2000;51: 245–270.
11. Danesh J, Wheeler JG, Hirschfield GM, et al. C reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med.* 2004;350:1387–1397.
12. Danesh J, Whincup P, Walker M, et al. Fibrin D dimer and coronary heart disease: prospective study and meta-analysis. *Circulation.* 2001; 103:2323–2327.
13. Tracy RP. Thrombin, inflammation, and cardiovascular disease: an epidemiologic perspective. *Chest.* 2003;124(3 suppl):49S–57S.
14. Lowe GD, Rumley A, McMahon AD, Ford I, O'Reilly DS, Packard CJ. West of Scotland Coronary Prevention Study Group. Interleukin-6, fibrin D-dimer, and coagulation factors VII and XIIa in prediction of coronary heart disease. *Arterioscler Thromb Vasc Biol.* 2004;24:1529–1534.
15. Lowe GD, Yarnell JW, Rumley A, Bainton D, Sweetnam PM. C-reactive protein, fibrin D-dimer, and incident ischemic heart disease in the Speedwell study: are inflammation and fibrin turnover linked in pathogenesis? *Arterioscler Thromb Vasc Biol.* 2001;21:603–610.
16. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The pathophysiologic roles of interleukin-6 in human disease. *Ann Intern Med.* 1998;128:127–137.
17. Robson SC, Shephard EG, Kirsch RE. Fibrin degradation product D-dimer induces the synthesis and release of biologically active IL-1 beta, IL-6 and plasminogen activator inhibitors from monocytes in vitro. *Br J Haematol.* 1994;86:322–326.
18. Tracy RP. Emerging relationships of inflammation, cardiovascular disease and chronic diseases of aging. *Int J Obes Relat Metab Disord.* 2003;27(suppl 3):S29–S34.
19. Jenny NS, Tracy RP, Ogg MS, et al. In the elderly, interleukin-6 plasma levels and the 174G>C polymorphism are associated with the development of cardiovascular disease. *Arterioscler Thromb Vasc Biol.* 2002;22:2066–2071.
20. Tracy RP, Lemaitre RN, Psaty BM, et al. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. Results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol.* 1997;17:1121–1127.
21. Cushman M, Lemaitre RN, Kuller LH, et al. Fibrinolytic activation markers predict myocardial infarction in the elderly. The Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol.* 1999;19:493–498.
22. Lutgendorf SK, Garand L, Buckwalter KC, Reimer TT, Hong SY, Lubaroff DM. Life stress, mood disturbance, and elevated interleukin-6 in healthy older women. *J Gerontol Med Sci.* 1999;54A:M434–M439.
23. Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, Atkinson C, Malarkey WB, Glaser R. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc Natl Acad Sci U S A.* 2003;100: 9090–9095.
24. von Känel R, Dimsdale JE, Adler KA, Patterson TL, Mills PJ, Grant I. Exaggerated plasma fibrin formation (D-dimer) in elderly Alzheimer caregivers as compared to noncaregiving controls. *Gerontology.* 2005;51:7–13.
25. Derogatis LR, Melisaratos N. The Brief Symptom Inventory: an introductory report. *Psychol Med.* 1983;13:595–605.
26. Pearlin LI, Mullan JT, Semple SJ, Skaff MM. Caregiving and the stress process: an overview of concepts and their measures. *Gerontologist.* 1990;30:583–594.
27. Holmbeck GN. Post-hoc probing of significant moderational and mediational effects in studies of pediatric populations. *J Pediatr Psychol.* 2002;27:87–96.
28. Kraemer HC, Wilson GT, Fairburn CG, Agras WS. Mediators and moderators of treatment effects in randomized clinical trials. *Arch Gen Psychiatry.* 2002:59:877–883.
29. Taaffe DR, Harris TB, Ferrucci L, Rowe J, Seeman TE. Cross-sectional and prospective relationships of interleukin-6 and C-reactive protein with physical performance in elderly persons: MacArthur studies of successful aging. *J Gerontol Med Sci.* 2000;55A:M709–M15.
30. Yarnell JW, Sweetnam PM, Rumley A, Lowe GD. Lifestyle factors and coagulation activation markers: the Caerphilly Study. *Blood Coagul Fibrinolysis.* 2001;12:721–728.
31. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K, Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. *J Clin Endocrinol Metab.* 1997;82:1313–1316.
32. Jerrard-Dunne P, Sitzer M, Risley P, et al. Interleukin-6 promoter polymorphism modulates the effects of heavy alcohol consumption on early carotid artery atherosclerosis: the Carotid Atherosclerosis Progression Study (CAPS). *Stroke.* 2003;34:402–407.
33. Nawawi H, Osman NS, Yusoff K, Khalid BA. Reduction in serum levels of adhesion molecules, interleukin-6 and C-reactive protein following short-term low-dose atorvastatin treatment in patients with non-familial hypercholesterolemia. *Horm Metab Res.* 2003;35:479–485.
34. Stevenson JC, Oladipo A, Manassiev N, Whitehead MI, Guilford S, Proudler AJ. Randomized trial of effect of transdermal continuous combined hormone replacement therapy on cardiovascular risk markers. *Br J Haematol.* 2004;124:802–808.
35. Lip GY, Lowe GD. Fibrin D-dimer: a useful clinical marker of thrombogenesis? *Clin Sci.* 1995;89:205–214.
36. von Känel R, Dimsdale JE. Fibrin D-dimer: a marker of psychosocial distress and its implications for research in stress-related coronary artery disease. *Clin Cardiol.* 2003;26:164–168.
37. Black PH. The inflammatory response is an integral part of the stress response: implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. *Brain Behav Immun.* 2003;17:350–364.
38. Jeanmonod P, von Känel R, Maly FE, Fischer JE. Elevated plasma C-reactive protein in chronically distressed subjects who carry the A allele of the TNF-alpha -308 G/A polymorphism. *Psychosom Med.* 2004;66:501–506.
39. Palareti G, Legnani C, Cosmi B, et al. Predictive value of D-dimer test for recurrent venous thromboembolism after anticoagulation withdrawal in subjects with a previous idiopathic event and in carriers of congenital thrombophilia. *Circulation.* 2003;108:313–318.
40. Blackwell K, Hurwitz H, Lieberman G, et al. Circulating D-dimer levels are better predictors of overall survival and disease progression than carcinoembryonic antigen levels in patients with metastatic colorectal carcinoma. *Cancer.* 2004;101:77–82.
41. Bush TL, Miller SR, Golden AL, Hale WE. Self-report and medical record report agreement of selected medical conditions in the elderly. *Am J Public Health.* 1989;79:1554–1556.
42. Kehoe R, Wu SY, Leske MC, Chylack LT Jr. Comparing self-reported and physician reported medical history. *Am J Epidemiol.* 1994;139:813–818.
43. Mukamal KJ, Kronmal RA, Tracy RP, Cushman M, Siscovick DS. Traditional and novel risk factors in older adults: cardiovascular risk assessment late in life. *Am J Geriatr Cardiol.* 2004;13:69–80.
44. von Känel R, Dimsdale JE, Ancoli-Israel S, et al. Poor sleep is associated with higher plasma proinflammatory cytokine interleukin-6 and procoagulant marker fibrin D-dimer in older Alzheimer caregivers. *J Am Geriatr Soc.* 2006;54:431–437.

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